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TITLE:

Identification and Manipulation of a Novel Signaling Mechanism To Improve Articular Cartilage Restoration After Posttraumatic Joint Injury

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14. ABSTRACT Injured articular cartilage evidences little intrinsic regenerative capacity. When healing does occur, either as a result of a full-thickness defect or microfracture surgery designed to mimic this event, mesenchymal cells from the bone marrow (BM-MSCs) produce disorganized collagen, with a pronounced bias toward collagen I (COL I) in lieu of collagen II (COL II). As a result, the newly-formed fibrocartilage has poor biomechanical properties and often evidences poor integration with the surrounding, native COL II-expressing cartilage, thus priming the tissue for subsequent degeneration. Identification of factors that drive fibrocartilage generation at the site of injury defines a new potential avenue of intervention in the field of cartilage restoration. We defined the lysophosphatidic acid (LPA)-autotaxin (ATX; encoded by the <i>ENPP2</i> gene) signaling axis as an important mediator of fibrocartilage formation both in vitro and in vivo. Addition of LPA to cultures of human chondrocytes and BM-MSCs substantially increased expression of COL I at the expense of COL II; this outcome could be reversed by small molecule or genetic inhibition of ATX activity. Importantly, drug-based inhibition of ATX activity led to reduced COL I expression and increased the secretion of COL II in a rat model similar to microfracture, thereby improving the quality of neocartilage formed after full-thickness injury.					
15. SUBJECT TERMS Scarring, fibrosis, joint injury, stiffness, fibrocartilage, lysophosphatidic acid					
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1 INTRODUCTION:

Injured articular cartilage evidences little intrinsic regenerative capacity. When healing does occur, either as a result of a full-thickness defect or microfracture surgery designed to mimic this event, mesenchymal cells from the bone marrow (BM-MSCs) produce disorganized collagen, with a pronounced bias toward collagen I (COL I) in lieu of collagen II (COL II). As a result, the newly-formed fibrocartilage has poor biomechanical properties and often evidences poor integration with the surrounding, native COL II-expressing cartilage, thus priming the tissue for subsequent degeneration. Identification of factors that drive fibrocartilage generation at the site of injury defines a new potential avenue of intervention in the field of cartilage restoration. Current study defines a new potential avenue of intervention in the field of cartilage restoration by targeting the lysophosphatidic acid (LPA)-autotaxin (ATX; encoded by the *ENPP2* gene) signaling axis as an important mediator of fibrocartilage formation both in vitro and in vivo.

2 KEYWORDS:

Scarring, fibrosis, joint injury, stiffness, fibrocartilage, lysophosphatidic acid, injury, cartilage

3 ACCOMPLISHMENTS:

What were the major goals of the project?

Goal 1 (out of 3 goals proposed for the entire project). To test whether LPA/ATX axis inhibition promotes chondrogenic differentiation and hyaline cartilage formation by bone marrow mesenchymal stem cells (BM MSC).

This goal was composed of 3 subtasks:

- 1a.** To determine the background levels of LPA in the culture medium used for chondrogenesis.
- 1b.** To create constructs carrying 3 shRNA sequences for stable silencing of the ATX gene expression.
- 1c.** To test chondrogenic differentiation of BM MSC with normal and silenced levels of the ATX expression.

To address these questions the following research activities have been carried out (please see quarterly reports for more specific information and additional data):

- have performed intensive analysis of multiple types of culture medium and bovine serum products to determine basal level of lysophosphatidic acid. This task allowed to generate an LPA-free/minimal culture system for all in vitro experiments to minimize
- generated stably transduced BM MSC using 4 lentiviral shRNA constructs targeting the ENPP2 gene.
- tested the chondrogenic potential of the control and transgenic BM MSC will be tested in an LPA-free/minimal microenvironment.

What opportunities for training and professional development has the project provided?

This project has offered a number of training opportunities for Dr Ling Wu, a postdoc responsible for majority of research and reporting activities. Dr Wu has been able to master new techniques for cell and molecular biology and also present his work at several international research meetings.

How were the results disseminated to communities of interest?

We have directly disseminated obtained data via direct communication with colleagues at professional meetings including International Cartilage Repair Society, American Society of Sport Medicine and the Orthopedic Research Society. Please see the list of conference presentations in Section 6 (Products).

What do you plan to do during the next reporting period to accomplish the goals?

During the next reporting period will focus on in vivo studies using rat model of joint surface injury. These studies will be instrumental for testing the effects of pharmacological inhibitors of the ATX/LPA axis during the process of healing and neochondrogenesis. In general this model mimics acute joint cartilage in humans. A pan-LPA inhibitor Brp-LPA will be loaded on PLGA (polylactic-glycolic acid) microspheres and delivered locally in the injured knee joint at the time of surgery. Next goal will also include in vivo assessment of chondrogenic potential of pluripotent stem cell-derived chondrocytes naturally expressing minimal/no LPA and BM MSC with silenced ATX activity and thus, minimal levels of LPA production. We predict that direct pharmacological inhibition of the ATX-LPA axis by the drug or implantation of cells with no or minimal levels of LPA will reduce the levels of fibrosis in the site of injury and promote hyaline cartilage formation.

4 IMPACT:

In our laboratory's preliminary work, we have identified a specific cellular pathway that is responsible for driving this disorganized scar formation. In this proposal, we aim to block this pathway following induced cartilage injuries in rats, and consequently diminish the production of scar tissue formed at the area of cartilage injury. If this work proves successful, it may be possible within a ~7-10 year time period to use simple, low-cost pharmacologic interventions to improve repair of injured cartilage without the use of donor tissue or extensive surgical reconstruction. This technique will be directly relevant to acute battlefield injuries of synovial joints.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5 CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

6 PRODUCTS:

Publications, conference papers, and presentations

Journal publications.

1. **Evseenko D**, Latour B, Richardson W, Corselli M, Sahaghian A, Zhu Y, Cardinal S, Chan R, Dunn B, and Crooks GM. (2013) PLOS ONE. Lysophosphatidic Acid Mediates Myeloid Differentiation within the Human Bone Marrow Microenvironment. 16;8(5):e63718. **NO**
2. Wu L, Gonzalez S, Shah S, Kyupelyan L Petrigliano FA, McAllister DR, Adams JS, Karperien M, Tuan T, Benya PD, **Evseenko D**. (2013) Extracellular matrix domain formation as an indicator of chondrocyte dedifferentiation and hypertrophy. Tissue Engineering Part C. 20(2):160-8. **YES**
3. Wu L, Latour B, Gonzalez S, Shah S, Kyupelyan L Galic Z, Van Handel B, Ge S, Zhu Y, Petrigliano FA, Li X, Lyons KM, Crooks GM, Adams JS, McAllister DR, **Evseenko D**. (2013) Human developmental chondrogenesis as a basis for the engineering of chondrocytes from pluripotent stem cells. Stem Cell Reports. 2;1(6):575-89. **YES**

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers, and presentations.

1. Wu L, Latour B, Gonzalez S Shah S, Kyupelyan L, Ge S, Petrigliano FA, McAllister DR, Li X, Adams JS, Crooks GM, Evseenko D. Molecular and functional characterization of primary cartilage cells at early stages of human development. UCLA 9th Annual Stem Cell Symposium, Los Angeles CA, 2013.
2. Wu L, Latour B, Gonzalez S Shah S, Kyupelyan L, Ge S, Petrigliano FA, McAllister DR, Adams JS, Benya P, Evseenko D. Functional cell sorting based on Extracellular Matrix Domain Detection (EMDD) UCLA 9th Annual Stem Cell Symposium, Los Angeles CA, 2013.
3. Wu L, Latour B, Gonzalez S, Shah S, Kyupelyan L, Ge S, Petrigliano FA, McAllister DR, Xinmin Li X, Adams JS, Crooks GM, Evseenko D. Human chondrogenesis as a basis for the engineering of chondrocytes. ISSCR 11 Annual Meeting, Boston, USA, 2013.

Website(s) or other Internet site(s)

Press release:

UCLA stem cell scientists first to track joint cartilage development in humans
<http://newsroom.ucla.edu/portal/ucla/PRN-ucla-scientists-first-to-track-249670.aspx>

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products

Nothing to report

7 PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Ling Wu
Project Role:	Postdoc
Researcher Identifier	N/A
Months	12
Contribution to Project:	<i>Dr Wu has performed all in vitro experiments for this study and has also presented research data at conferences</i>
Funding Support:	N/A

Name:	Josh Lee
Project Role:	Technician
Researcher Identifier	N/A
Months	6
Contribution to Project:	<i>Dr Lee has assisted Dr Wu in most of the reported experiments and was responsible for the maintenance of cell cultures, vector packaging, ordering and basic molecular techniques</i>
Funding Support:	<i>6 months: California Institute of Regenerative Medicine (CIRM)</i>

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

None

8 SPECIAL REPORTING REQUIREMENTS

Collaborative awards:

N/A

Quad charts:

N/A

9. APPENDICES

N/A